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Human CP-IgA (chlamydia pneumoniae-Immunoglobulin A) ELISA Kit

Product Overview

Name	Human CP-IgA (chlamydia pneumoniae-Immunoglobulin A) ELISA Kit
Sensitivity	Qualitative
Sample type(s)	Serum, Plasma

Principle of the Assay

This kit was based on indirect ELISA. CP-Ag was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP- Conjugates, if there were any CP-IgA in the samples, it would form a CP-Ag- CP-IgA- HRP- Conjugates complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (?1 month) or -80°C (?2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Material Required But Not Supplied

1.Microplate reader (wavelength: 450nm)

2.37°C incubator

3.Automated plate washer

4.Precision single and multi-channel pipette and disposable tips

5.Clean tubes and Eppendorf tubes

6.Deionized or distilled water

Assay procedure

1. Label the sample wells, 3 Negative Controls, 2 Positive Controls and 1 blank wells.

2. Add 100?L Negative Controls and Positive Controls to each wells.

3. Add 100?L sample dilution buffer to sample wells and then add 10?L sample serum or plasma. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37? for 30min.

4. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1 2 minute each time.

5. Add 100 µL HRP- Conjugates to each well, except blank well

6. Seal the plate with a cover and incubate at 37? for 30 min.

7. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1 2 minute each time.

8. Add 50 ?l of TMB substrate A and 50 ?l of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37? in dark within 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

9. Add 50 ?l of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

10. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)